Radioresistant Rag2-IL2rg (R2G2) mice demonstrate position-dependent bioluminescent tumor signal and increased lethality compared to severe combined immunodeficient (SCID) mice in a disseminated lymphoma model.

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Abstract

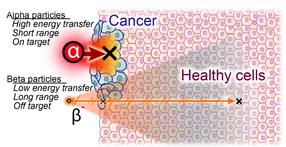
Radioimmunotherapy (RIT) offers hope for treatment-refractory non-Hodgkin lymphoma (NHL) by targeting radiation to CD20-expressing cells. However, current lymphoma models typically use mice with DNA repair deficiencies (i.e., SCID mice), thereby potentially limiting RIT doses in pre-clinical studies. To overcome this, **our objective was to establish a disseminated lymphoma model using the relatively radioresistant Rag2-IL2rg (R2G2) double-knockout mouse strain** with intact double-stranded DNA repair. We assessed survivorship and NHL-cell tumor growth between R2G2 mice and the more commonly used athymic nude and SCID mice after intravenous delivery of 1 million Raji lymphoma cells stably transfected with the luciferase reporter gene (Rajj-luc). All athymic nude mice survived, but R2G2 mice had decreased median survival time compared to SCID mice (17 vs. 32 days; p<0.001, log-rank test). Bioluminescence (BLI) signal increased over time in both strains, but did not differ between R2G2 and SCID mice at day 13 post-injection if animals were imaged prone (p=0.37, unpaired t-test). However, when mice were imaged supine, R2G2 BLI signal was 17.3-fold greater (p<0.001, unpaired t-test) compared to SCID mice. Consistent with these results, Raji-luc BLI signal attenuation by mouse pelts was dependent on the strain type and pelt location, with R2G2 pelts showing the greatest attenuation between strains and with greater attenuation seen by the brown R2G2 dorsal pelt than with the light tan R2G2 ventral pelt (p<0.001, ANOVA with multiple comparisons). We found positional dependence of optical imaging, with supine imaging providing a more accurate representation of disease burden in R2G2 mice than prone imaging, likely due to the lighter coat color on the ventral surface. **Given the preservation of double-stranded DNA-break repair mechanisms, R2G2 mice may be an excellent strain for translational studies with RIT.**

Why study lymphoma in the context of radioimmunotherapy?

In 2020, lymphoma will cause over 20,000 deaths and cost over $20 billion annually in the US.

Treatment-refractory non-Hodgkin lymphoma (NHL) will account for most of this mortality and expense.

Radioimmunotherapies (RITs), which use CD20-targeting monoclonal antibodies (mAbs) linked to radioisotopes, improve efficacy in lymphoma treatment by selectively killing CD20-expressing cancer cells with radiation.



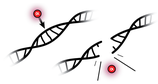
Targeted alpha particle therapy (**TAT**) treats cancer by bringing alpha-emitting radionuclides in proximity of target cells and has a high potential for use in lymphoma.

Targeted alpha particle (TAT) therapy offers advantages over currently used beta particles. Compared to beta radiation, alpha particles deliver radiation with much greater linear energy transfer with improved cytotoxicity over a shorter range and minimizing crossfire damage to nearby normal cells.

Current lymphoma models are limited for assessing therapies.

SCID mice are commonly used as a model to study lymphoma.

SCID mice have disrupted DNA repair mechanisms.



Many therapies, including alpha particle therapy, disrupt DNA.

Because healthy cells have defective DNA repair, SCID mice poorly tolerate relatively low radiation doses making them a suboptimal model for studying targeted alpha particle therapy.

To overcome this limitation we explored alternative mouse strains.

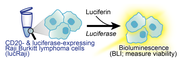
What are the differences between mouse strains in the current study?

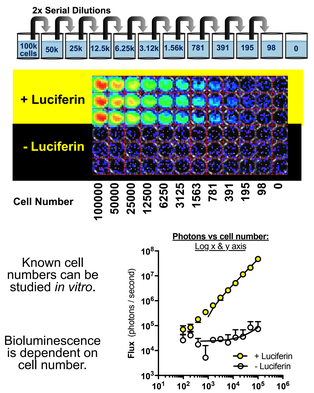
Strain	Coat color	Defect	Phenotype	Radiosensitivity
SCID 	Dorsal / Ventral 	Autosomal recessive **Mutated *Prkdc* gene** Protein Kinase, DNA-Activated, Catalytic Polypeptide Molecular sensor for DNA damage	**Immunodeficient** Decreased adaptive immunity Impaired T & B cell development. Deficient in T & B cells. Normal NK cells, macrophages, and neutrophils.	HIGH 
Nude 	Dorsal / Ventral 	Autosomal recessive ***nu* allele of Foxn1** Foxn1 = Forkhead Box N1 Encodes a transcription factor	**Mildly-immunodeficient** Dysfunctional rudimentary thymus T-cell deficient No generation of cytotoxic effector cells B-cells function normal Hairless	Low 
R2G2 	Dorsal / Ventral 	**Recombination activating gene 2 (*Rag2*)** Part of a protein complex that breaks DNA Essential for making mature T & B cells **Common gamma chain gene (*Il2rg*)** Co-receptor in many IL receptors	**Super-Immunodeficient** Decreased innate AND adaptive immunity Severe lymphocyte development impairment Deficient in T & B cells, Lack NK cells Lacks viable receptors for IL-2, -4, -7, -9 & IL-15 Low macrophages, dendritic cells, & neutrophils	Low 

How do we study disseminated lymphoma in mice?

1) We use luciferase-expressing Raji lymphoma cells

Luciferase-expressing Raji cells produce light (bioluminescence) which can be used to quantify the number of living cells.





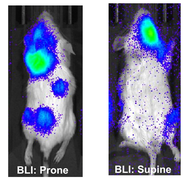
Known cell numbers can be studied *in vitro*.

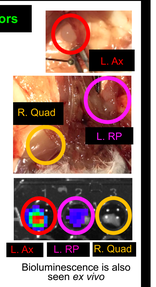
Bioluminescence is dependent on cell number.

2) Bioluminescence is seen in discrete tumors

Disseminated lymphoma is established with intravenous tumor cell injection

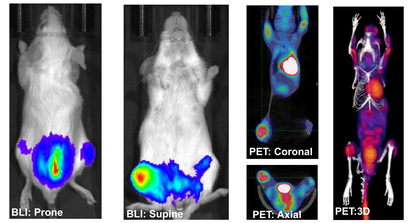
Bioluminescence is seen after luciferin delivery



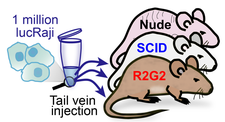


Bioluminescence is also seen *ex vivo*

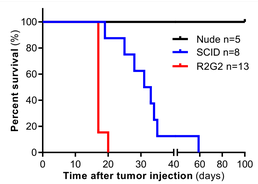
3) Bioluminescence registers with FDG-PET activity



Do mice strains differ in survival after delivery of lymphoma cells?



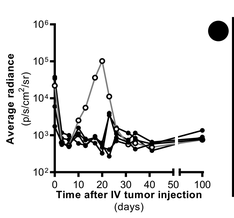
We compared survivorship in nude, SCID, and R2G2 mice after delivery of 1,000,000 lucRaji lymphoma cells IV.

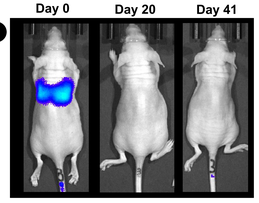


P < 0.001
log-rank mantel-cox.

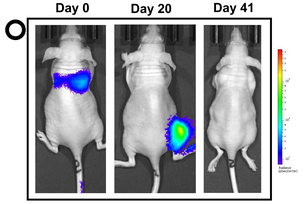
R2G2 mice demonstrate markedly decreased survivorship compared to SCID mice and nude mice despite equal initial cell number delivery.
All nude mice survived.

Why do nude mice survive?





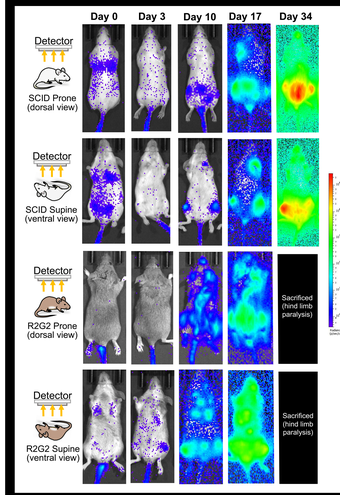
Nude mice typically initially show tumor in lungs after IV injection with subsequent spontaneous clearance.



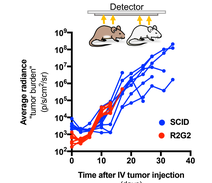
One mouse had tumor present in the right knee, which subsequently cleared.

Tumor is cleared from nude mice.

Does tumor bioluminescence signal differ between strains *in vivo*?

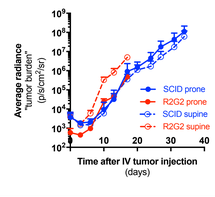


When mice are imaged **prone**, there is similar bioluminescence between **SCID** and **R2G2** mice.

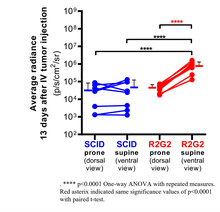


When mice are imaged **supine**, there is increased bioluminescence in **R2G2** mice.

Combined and averaged data for mouse strains and position



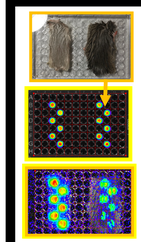
Comparison of position and mouse strain on Day 13



**** p<0.0001 One-way ANOVA with repeated measures. Red asterix indicated same significance values of p<0.0001 with paired t-test.

Bioluminescence signal is increased when R2G2 mice are imaged supine (ventral view).

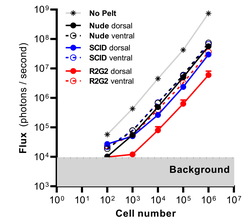
Do pelt differences affect tumor bioluminescence signal?

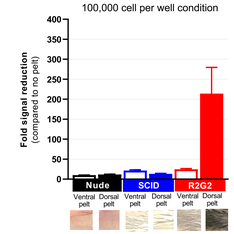


Remove ventral & dorsal pelts post mortem

Overlay wells containing lucRaji cells

Measure simulated tumor bioluminescence





The R2G2 dorsal pelt markedly attenuates tumor bioluminescence.

Summary

This work provides critical information for choosing the appropriate mouse strain for studying RIT in NHL. Athymic nude mice spontaneously cleared lymphoma cells, precluding use in RIT studies. Conversely, tumor growth progressed and lethality was reached in all R2G2 and SCID mice. The survival time in R2G2 mice was nearly half of that in SCID mice, likely secondary to greater immunodeficiency in R2G2 mice. Furthermore, we found positional dependence of optical imaging, with supine imaging providing a more accurate representation of disease burden in R2G2 mice than prone imaging, likely due to the lighter coat color on the ventral surface. **R2G2 mice may be optimal for translational studies with RIT given the preservation of double-stranded DNA-break repair mechanisms.**

References

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